CHAPTER 2

LITERATURE REVIEW

2.1 Botanical description of *Senna alata*

Senna alata (L.) Roxb. (previously name *Cassia alata*) is a medicinal plant in Leguminosae family (Figure 2-1). It has many common names such as: Candel bush, Acapulo, Ringworm bush, and Calabra bush. In Thai, it is so called Chum-Het-Thet (central Peninsular), Kheekhaak, Lapmuen luang, Maak kaling thet (Northern), Chumhet yai (Central), and Ta-see pho (Karen-Mae Hong Son) (เต็ม สมิตินันท์, 2544).

Figure 2-1 *Senna alata* (L.) Roxb.

The plant is a shrub normally 1 - 2 m high but sometimes up to 5 m high and has horizontally spread branches. Leaves are paripinnate, 30 - 60 cm long; consisting of 8 - 20 pair of leaflets, each leaflet is oblong or elliptic oblong, rounded at both, ends, 5 - 15 by 3 - 7 cm, glabrous. The petioles are robust, 2 - 3 mm long. Flowers are densely in axillary racemes, about 20 - 50 cm long and 3 - 4 cm broad. The bracts are caduceus, 2 - 3 by 1 - 2 cm broad. The pedicels are very short, about 2 - 4 m long. There are 5, unequal, oblong, 10 - 20 by 6 - 7 mm, green sepals. The petals are bright yellow, ovate-orbicular to spathulate, short-clawed, 2 by 1 - 1.5 cm. There are 9 - 10 stamens; 2 large, 4 small and 3 - 4 stamens are reduced. The anthers are opening by apical pores. There is only one pistil and glabrous ovary. Fruit is a thick, flattened, wing, glabrous pod, 10 - 15 by 1.5 - 2 cm. The wings are 5 mm broad. Seed are about 50, flattened, more or less quadrangular, 7 - 10 by 5 - 8 mm and black (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998).

Senna alata grows well in full sun in a wide range of soils, which retain moisture adequately. The plants grow in waste places, often along ditches between rice-fields. The plants are usually propagated by seeds and distributed all over the country up to 1,500 m above sea level; sometimes they are cultivated for medical purposes (Farnsworth and Bunyapraphatsara, 1992).

2.2 Thai traditional uses of *S. alata*

In Thailand, *S. alata* leaves and flowers have long been traditionally used as laxative and antifungal agents (Farnsworth and Bunyapraphatsara, 1992).

2.2.1 Use as laxative

1. A few flowers (3 inflorescences) are cooked in boiling water and taken with a special sauce called “Namprik” or taken as fresh flowers.

2. Eight to twelve leaves are sun dried and powder. An in fusion is made from the powdered leaves and taken before bedtime.

3. Three to five branches with leaves are boiled with water (1,500 ml). The decoction is boiled until about one third of water used is obtained. Salt is added to the infusion to give it a salty taste. One glass of the decoction is taken.

4. Twelve fresh or dried leaves are boiled in water (250 ml), and then the water extract is taken before bed time.

2.2.2 Treatment of ringworm or Tinea versicolor

1. Fresh leaves are pounded and the juice obtained is applied over the infected area.

2. Three to four fresh leaves are pounded. Lemon juice is then added. The obtained juice is applied over the infected area.

2.3 Ethnomedical uses

The claim efficacies in Thai ethnomedical textbook are as follows (Farnsworth and Bunyapraphatsara, 1992).

Stems	Treatment of yaws, ringworm, Tenea versicolor, constipation, urinary stone, anthelmintic and cardiotonic
Leaves	Treatment of skin diseases, urinary stone, ringworm, Tenea versicolor, laxative, cardiotonic and expectorant
Flowers	Laxative and improvement of appearance and texture of skin
Pod	As an anthelmintic
Seed	Treatment of skin diseases, constipation and as an anthelmintic
Whole plant	As an anthelmintic and antipyretic
Not specific part	Treatment of skin diseases, haemorrhoids, chronic gastrointestinal aliments of children between the ages of 5 and 13 years characterized by marked malnutrition, usually associated with intestinal parasitism.

2.4 Chemical constituents of *S. alata*

Phytochemical studies of *S. alata* have been reported that it contains variety of secondary compounds including anthraquinones, flavonoids, sterols, tannins, triterpenoids, saponins and fatty acid as shown in Table 2-1.

Table 2-1 Chemical constituents of various parts of *S. alata*

Plant part	Category	Chemical substance	Reference
Leaves	Flavonoid glycoside	kaempferol-3-*O*-gentiobioside	Moriyama *et al*., 2003
	Flavonoid	kaempferol	Rao *et al*., 1975
	Anthraquinone	chrysophanol	Morah and Otumu, 1991
		emodin	Morah and Otumu, 1991
		aloe-emodin	Morah and Otumu, 1991
		rhein	Morah and Otumu, 1991
		isochrysophanol	Smith and Sadaquat, 1979
	Anthraquinone glycoside	rhein-8-glucoside	Rai, 1978
		aloe-emodin-8-glucoside	Rai, 1978
		sennoside A, B, C, and D	Harrison and Garro, 1997
		physcicon-L-glucoside	Smith and Sadaquat, 1979
	Polyphenol	2,3,7-tri-*O*-methylellagic alquinone	Alam *et al*., 2003
Root	Anthraquinone		Yadav and Kalidhar, 1994
Stem	Flavonoid glycoside	kaempferol-3-*O*-gentiobioside	Moriyama *et al*., 2003
	Anthraquinone	emodin	Kelly *et al*., 1994
		1,5-dihydroxy-2-methylanthraquinone	Rai and Prasad, 1994

Table 2-1 (cont.)

Plant part	Category	Chemical substance	Reference
		5-hydroxy-2-methylanthraquinone-1-*O*-rutinoside	Rai and Prasad, 1994
	Anthrone	3-formyl-1,6,8,10-tetrahydroxyanthrone (alarone)	Hemlata and Kalidhar, 1994.
	Sterol	*β*-sitosterol	Rai and Prasad, 1994
Fruit	Anthraquinone	rhein	Rai, 1978
		aloe-emodin	Rai, 1978
		emodin	Rai, 1978
Seed	Polyalcohols	glycerol	Singh, 1998
		erythritol	Singh, 1998
	Carbohydrate	galactomannans	Gupta *et al*., 1987
	Flavonoid glycoside	chrysoeriol-7-*O*-(2"-*O*-*β*-D-mannopyrannosyl)-*β*-D-allopyranoside	Dipti, 1991
		rhamnetin-3-*O*-(2"-*O*-*β*-D-mannopyrannosyl)- *β*-D-allopyranoside	Dipti, 1991
	Sterol	*β*-sitosterol	Miralles and Gaydou, 1986
		sitostrol	Singh and Tiwari, 1943
		stigmasterol	Miralles and Gaydou, 1986
		campesterol	Miralles and Gaydou, 1986
		22-dihydrospinasterol	Miralles and Gaydou, 1986
		28-isoavenasterol	Miralles and Gaydou, 1986
	Fatty acid	linoleic acid	Singh and Tiwari, 1943

Table 2-1 (cont.)

Plant part	Category	Chemical substance	Reference
		oleic acid	Singh and Tiwari, 1943; Morah and Otumu, 1991
		palmitic acid	Singh and Tiwari, 1943; Morah and Otumu, 1991
		lignoceric acid	Singh and Tiwari, 1943
		isopalmitic acid	Morah and Otumu, 1991
		palmitoleic acid	Morah and Otumu, 1991
		myristoleic acid	Morah and Otumu, 1991
		tridecanoic acid	Morah and Otumu, 1991
		myristic acid	Morah and Otumu, 1991
	Anthraquinones	rhein	Morah and Otumu, 1991
		aloe-emodin	Morah and Otumu, 1991
		emodin	Morah and Otumu, 1991
		chrysophanol	Morah and Otumu, 1991

2.5 Determination of hydroxyanthracene derivatives (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998)

Total content of hydroxyanthracene derivatives in the plant materials was measured by a spectrophotometric method according to the Thai Herbal Pharmacopoeia (1998), as follows; About 150 mg of the leaf powder was accurately weighed and placed in a 100 ml round bottom flask. A portion of water (30 ml) was added, mixed, weighed, and heated under a reflux condenser for 15 minutes. The cooled mixture was weighed and adjusted to the original weight with water. The mixture was centrifuged and the supernatant liquid (20 ml) was transferred to a 150 ml separator. 2 M hydrochloric acid (0.1 ml) was added and the mixture was shaken with three 15 ml portions of chloroform. The chloroform layer was discarded. Sodium hydrogen carbonate (100 mg) was added into the aqueous part and shaken for 3 minutes. After

centrifugation, the supernatant liquid (10 ml) was transferred to a 100 ml round bottom flask. 10.5 %w/v solution of iron (III) chloride (20 ml) was added and the mixture was heated for 20 minutes under a reflux condenser. Hydrochloric acid (1 ml) was added and heated for a further 20 minutes with frequent shaking. After cooling, the mixture was transferred to a separator and shaken with three 25 ml portions of ether previously used to rinse the flask. The ether layers were combined and washed with two 15 ml portions of water. The ether layer was then transferred to a 100 ml volumetric flask and diluted with ether to the required volume. An aliquot of the solution (25 ml) was carefully evaporated to dryness at low temperature and the residue was dissolved in 10.0 ml of a 0.5 %w/v solution of magnesium acetate in methanol. The absorbance of the solution was measured by SPECTRO UV-VIS RS Spectrophotometer at 515 nm, using the magnesium acetate solution as the blank. The percentage of rhein-8-glucoside was calculated from the expression: A × 0.4283/w, where A is the absorbance measured finally at 515 nm, and w is the weight in g of the dried leaf powder used initially.

2.6 Biological activities of *S. alata* extracts

Several studies on the biological activities of *S. alata* extracts have been reported. The crude extract of *S. alata* leaf was examined for antimicrobial activities by disc diffusion and broth dilution methods and found that it had strong inhibitory effect against *Propionibacterium acnes* and *Staphylococcus epidermidis* with the MIC values of 0.625 and 2.5 µg/ml, respectively (Chomnawang *et al*., 2005).

The crude ethanol and water extracts of *S. alata* leaves and barks were evaluated antimicrobial activities against fungi (*Aspergillus fumigatus* and *Microsporum canis*), yeast (*Candida albicans*) and bacteria (*S. aureus* and *E. coli*). Both ethanol and water extracts of *S. alata* barks, but not *S. alata* leaves, showed concentration-dependent antifungal activity against *C. albicans*. The water extract from the barks showed bigger inhibition zone than the ethanol extract. In contrast, neither of the tested extracts was active against *A. fumigatus* and *M. canis*. For antibacterial activity testing, only water and ethanol extracts of *S. alata* leaves exhibited

antibacterial activity against *S. aureus*. In addition, neither of tested extracts was active against *E. coli* (Somchit *et al*., 2002).

The methanol extracts of leaves, flowers, stem and root barks of *S. alata* have also been reported as broad spectrum antibacterial agents. The antibacterial activity was improved by fractionation with the various organic solvents, such as petrol, dichloromethane and ethyl acetate. The dichloromethane fractions of the flower extract exhibited the highest antibacterial activity. However, the antifungal activity of all tested extracts was not observed (Khan *et al*., 2000).

The ethanol extract of *S. alata* leaves was investigated for its antimicrobial activities against several microorganisms including bacteria, yeast, dermatophytic fungi and non-dermatophytic fungi. The extract exhibited antifungal activity against various species of derrnatophytic fungi with the MIC value of 125 mg/ml for *Trichophyton mentagorphytes* var. *interdigitale*, *Trichophyton rubrum* and *Microsporum gypseum* and the MIC value of 62.5 mg/ml for *Microsporum canis*. However, the extract showed low antifungal activity against non-dermatophytic fungi. The mechanism of inhibition may be related to the cell leakage as observed by irregular, wrinkle shape and loss in rigidity of the macroconidia (Ibrahim and Osman, 1994). In contrast, the extract was not effective against bacterial and yeast species. In addition, It has also been reported that the methanol extract of *S. alata* leaves exhibited antifungal activities against three pathogenic fungi including *M. gypseum*, *T. rubrum* and *Penicillium marneffei* with the 50% inhibition concentration (IC_{50}) of hyphal growth of 0.5 and 0.8 mg/ml against *T. rubrum* and *M. gypseum*, respectively (Phongpaichit *et al*., 2004).

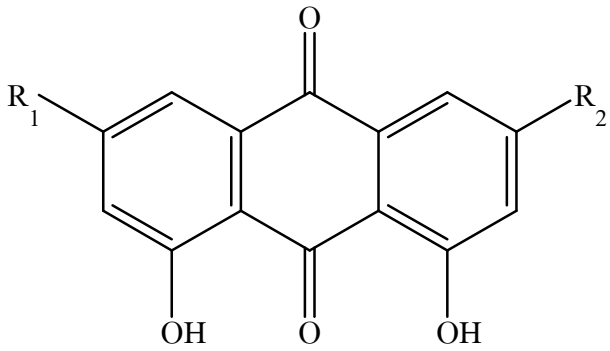
It has been reported that using of the ointments made from ethanolic extracts of *S. alata* leaves as topical treatments for chronic crusty or acute lesions of dermatophilosis induced healing of the disease without recurrence in nine treated animals. These ointments, when applied once a day for 8-15 days, provoked the falling off of the crusts after 3-4 days of treatment. Hair grows on the treated areas, which heal without scarring, within 3-4 weeks after the end of the

treatment. The healed animals became free of dermatophilosis without recurrence for more than 3 years and were in good health (Emmanuel *et al*., 2003).

It has been reported that the crude *S. alata* extracts, containing steroids, anthraquinone glycosides, volatile oils and tannins, exhibited a high MIC value of 500 mg/ml against *S. aureus*, *Streptococcus faecalis*, *Micrococcus luteus*, *Bacillus. subtilis* and *Pseudomonas putida*, but was generally inactive against *E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Pseudomonas fluorescens* (concentration higher than 1000 mg/ml) (Adedayo *et al*., 2001)

The methanol extract of *S. alata* leaves showed higher antifungal activity than the ethanol and petroleum ether extracts also has been reported. The unidentified active components purified from preparative thin layer chromatography exhibited low activities against *Mucor, Rhizopus* and *Aspergillus niger* at 70 μg/ml while higher activity was exhibited against all the test organisms at 860 μg/ml (Owoyale *et al*., 2005).

It has been reported that the antifungal activity of *S. alata* was related to anthraquinones (Singh *et al*., 2006) (Figure 2-2).



Aloe-emodin	:	$R_1 = CH_2OH$; $R_2 = H$
Chrysophanol	:	$R_1 = CH_3$; $R_2 = H$
Emodin	:	$R_1 = CH_3$; $R_2 = OH$
Physcione	:	$R_1 = CH_3$; $R_2 = OCH_3$
Rhein	:	$R_1 = COOH$; $R_2 = H$

Figure 2-2 Chemical structures of anthraquinones

The methanolic extract of *S. alata* leaves also exhibited a stronger antioxidant activity (ED_{50} 28.50 ± 1.86 µg/ml) than the extracts from the flowers (ED_{50} 175.36 ± 2.07 µg/ml) and pods (ED_{50} 100.18 ± 2.59 µg/ml), on the basis of DPPH radical scavenging assay. The antioxidative constituent was isolated and identified as kaempferol (Panichayupakaranant and Kaewsuwan, 2004).

2.7 Biological activity of anthraquinones

2.7.1 Aloe-emodin

Aloe-emodin isolated from *Rheum emodi* rhizomes exhibited antifungal activity against *C. albicans* and *T. mentagrophytes* with the MIC values of 50 µg/ml (Agarwal *et al*., 2000).

The capacity of aloe-emodin to reduce the cytotoxicity of the proinflammatory cytokine tumor necrosis factor (TNF) towards L929 mouse fibrosarcoma and U251 human glioma cell lines has been demonstrated (Harhaji *et al*., 2007). Aloe emodin inhibited both TNF-induced cell necrosis and apoptosis, but it did not reduce cell death induced by UV radiation or hydrogen peroxide. Aloe-emodin inhibited both basal and TNF-triggered activation of extracellular signal-regulated kinase (ERK). A selective blockade of ERK activation mimicked the cytoprotective action of the drug. On the other hand, aloe-emodin did not affect TNF-induced activation of p38 mitogen-activated protein kinase or generation of reactive oxygen species. The combination of aloe-emodin and TNF caused an intracellular appearance of acidified autophagic vesicles, and the inhibition of autophagy with bafilomycin or 3-methyladenine efficiently blocked the cytoprotective action of aloe-emodin. These data indicate that aloe-emodin could prevent TNF-triggered cell death through mechanisms involving induction of autophagy and blockade of ERK activation.

Aloe-emodin inhibited cancer cells in a dose-dependent manner. Treatment with aloe-emodin at 10 to 40 µM resulted in cell cycle arrest at G2/M phase. The alkaline phosphatase (ALP) activity in KB cells increased upon treatment with aloe-emodin when compared to controls. This is one of the first studies to focus on the expression of ALP in human oral carcinomas cells treated with aloe-emodin. These results indicate that aloe-emodin has anti-cancer effect on oral cancer, which may lead to its use in chemotherapy and chemopreventment of oral cancer (Xiao *et al*., 2006).

Aloe-emodin-induced CH27 cell apoptosis was confirmed by DNA fragmentation (DNA ladders and sub-G1 formation). Aloe-emodin-induced apoptosis of CH27 cells involved modulation of the expression of Bcl-2 family proteins, such as $BclX_L$, Bag-1, and Bak, and was associated with the translocation of Bak and Bax from cytosolic to particulate fractions. Aloe-emodin treated CH27 cells had an increased relative abundance of cytochrome *c* in the cytosolic fraction. Results demonstrated that the activation of caspase-3, caspase-8, and caspase-9 is an important determinant of apoptotic death induced by aloe-emodin. These results suggest that aloe-emodin induces CH27 cell death by the Bax and Fas death pathway (Lee *et al*., 2001).

Aloe-emodin induced DNA single strand breaks were observed by comet assay. Aloe-emodin induced decreases in the mRNA of DNA repair enzymes such as hMTH1, hOGG1 and APE. Although the activity of the radical-scavenging enzyme SOD was enhanced by aloe-emodin, the effects of aloe-emodin on H460 cell apoptosis were suspected to result from the prooxidant. These results suggest that aloe-emodin induced DNA damage through generation of reactive oxygen species in human lung carcinoma cells (Lee *et al*., 2005).

Results from flow cytometry demonstrated that aloe-emodin delayed the number of cells entering and exiting DNA synthesis (S) phase in both SVG and U-373MG cells indicating that aloe-emodin may inhibit S phase progression. Assessment of cell viability demonstrated that SVG and U-373MG glioma cell were highly sensitive to aloe-emodin. The aloe-emodin-induced decreased proliferation was sustained at 48-96 h. A PKC activity assay was quantified to establish the role of PKC in aloe-emodin's mode of action. Exposure of SVG and U-373MG glioma cells to aloe-emodin suppressed PKC activity and reduced the protein content of most of the PKC isozymes. The results indicate the cancer growth inhibition by aloe-emodin (Acevedo *et al*., 2004).

Flow cytometric assays and DNA fragmentation gel electrophoresis also confirmed aloe-emodin induced apoptosis in HL-60 cells. The levels of caspase-3 were increased after HL-60 cells were co-treated with 10 μM aloe-emodin for 12, 24, 48, and 72 hours. Taken

together, aloe-emodin therefore appears to exert its anti-carcinogenesis properties by inhibiting proliferation and inducing cell cycle arrest and apoptosis underwent activation of caspase-3 in human leukemia HL-60 cells (Chen *et al*., 2004).

2.7.2 Rhein

Rhein is an anthraquinone compound enriched in the rhizome of rhubarb, a traditional Chinese medicine showing anti-tumor promotion function. Rhein could induce apoptosis in human promyelocytic leukemia cells (HL-60), characterized by caspase activation, poly (ADP) ribose polymerase (PARP) cleavage, and DNA fragmentation. The efficacious induction of apoptosis was observed at 100 μM for 6 h. Mechanistic analysis demonstrated that rhein induced the loss of mitochondrial membrane potential ($\Delta\Psi_m$), cytochrome c release from mitochondrion to cytosol, and cleavage of Bid protein. Rhein also induced generation of reactive oxygen species (ROS) and the phosphorylation of c-Jun N-terminal kinase (JNK) and p38 kinase. However, these actions seem not to be associated with the apoptosis induction because antioxidants including N-acetyl cysteine (NAC), Tiron, and catalase did not block rhein-induced apoptosis, although they could block the generation of ROS and the phosphorylation of JNK and p38 kinase. The data demonstrate that rhein induces apoptosis in HL-60 cells via a ROS-independent mitochondrial death pathway (Lin *et al*., 2003).

Rhein isolated from *Rheum emodi* rhizomes exhibited antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *T. mentagrophytes* with the MIC values of 50, 50, and 25 μg/ml, respectively (Agarwal *et al*., 2000).

Rhein also inhibited pyrogallol auto-oxidation and showed free-radical scavenging activity against hydroxyl radical (Yuan and Gao, 1997).

2.7.3 Emodin

Emodin also exhibited antibacterial activity against *B. subtilis* and *S. aureus* with the MIC values of 7.8×10^{-3} and 3.9×10^{-3} mg/ml, respectively. However, it was not active against two Gram-negative bacteria (*Klebsiella pneumoniae* and *E. coli*) at the highest concentration (5.0×10^{-1} mg/ml) tested (Chukwujekwu *et al*., 2005).

Emodin significantly suppressed IL-1β induced MC proliferation and arrested the cell-cycle progress *in vitro*. Fibronectin and collagen IV production by MC were significantly reduced after emodin treatment. No alterations of P38 expression and PMKK4 protein content were observed. However, protein levels of P-P38 and P-MKK3/6 significantly decreased after emodin treatment. In the renal failure models, after administration of emodin for eight weeks, the rat renal lesions were significantly ameliorated, as evidenced by the decreased blood creatinine, urea, and the 24-hour urine protein. The results indicate that emodin suppresses IL-1β induced MC proliferation and ECM production *in vitro*. Emodin ameliorates renal failure in subtotal nephrectomized rats, which suggests a potential role of emodin in the treatment of progressive renal diseases (Wang *et al*., 2007). Emodin was also efficient to ameliorate renal dysfunction in diabetic nephropathy rats probably by its inhibition of the activation of p38 MAPK pathway and downregulation of the expression of fibronectin (Wang *et al*., 2006).

Emodin significantly blocked the S protein and ACE2 interaction in a dose-dependent manner. It also inhibited the infectivity of S-protein-pseudotyped retrovirus to Vero E6 cells. These findings suggested that emodin may be considered as a potential lead therapeutic agent in the treatment of severe acute respiratory syndrome (SARS) (Ho *et al*., 2006).

It has been reported that emodin promoted repair of rats' excisional wounds via a complex mechanism involving stimulation of tissue regeneration and regulating Smads-mediated TGF-β_1 signaling pathway (Tang *et al*., 2007).

Effects of emodin treatment at 3 daily doses (0.6 or 1.2 mmol/kg) could enhance myocardial mitochondrial ATP generation capacity and antioxidant components in both male and female rat hearts, but it only significantly protected against I-R injury in female hearts. Treatment with a single dose of emodin invariably enhanced mitochondrial antioxidant components and protected against I-R injury in both male and female hearts. The gender-dependent effect of emodin treatment at multiple doses may be related to the differential antioxidant response in the myocardium and/or induction of drug metabolizing enzymes in the liver (Do and Ko, 2005).

It has been reported emodin significantly inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced *in vitro* invasion of human cancer cells including HSC5 and MDA-MB-231 cells. Matrix metalloproteinases (MMPs) are known to be associated with cancer invasion. Zymographic analysis showed that emodin suppressed TPA-induced MMP-9 activity in a concentration dependent manner. In addition, emodin reduced the transcriptional activity of activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB), two important nuclear transcription factors involved in MMP-9 expression. Emodin suppressed the phosphorylation of two mitogen-activated protein kinases, extracellular signal-regulated protein kinase and c-Jun N-terminal kinase, but not p38 kinase, leading to reduced c-Jun phosphorylation and AP-1 DNA-binding. Moreover, emodin inhibited TPA-induced degradation of inhibitor of kappa Bα, nuclear translocation of p65, and NF-κB DNA-binding activity. These results suggest that emodin inhibits the invasiveness of human cancer cells by suppressing MMP-9 expression through inhibiting AP-1 and NF-κB signaling pathways (Huang *et al*., 2004).

The inhibitory effect of emodin on the direct-acting mutagenicity of 1-nitropyrene (l-NP) has been examined using the Ames/microsomal test with *Salmonella typhimurium* TA98 and the genotoxicity of l-NP has been evaluated using the SOS chromotest with *E. coli* PQ37. Emodin decreased the mutagenicity of l-NP in a dose-dependent manner in both assay systems. Furthermore, emodin significantly inhibited the formation of l-NP DNA adducts in *S. typhimurium* TA98 in the ^{32}P-postlabeling study. The results suggest emodin act as blocking and/or suppressing agents to reduce the direct-acting mutagenicity of l-NP (Su *et al*., 1995).

2.7.4 Chrysophanol

Chrysophanol isolated from *Rheum emodi* rhizomes exhibited antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *T. mentagrophytes* and *Aspergillus fumigatus* with the MIC values of 50, 50, 25, and 50 µg/ml, respectively (Agarwal *et al*., 2000).

Chrysophanol has also been tested for plant disease control activity *in vivo* against six plant pathogenic fungi. The concentrations required for 50% disease control was 4.7 mg/ml for chrysophanol. These agents showed both curative and protective activity against barley powdery mildew. Chrysophanol (100 mg/ml) was more effective than the fungicides fenarimol (30 mg/ml) and polyoxin B (100 mg/ml), under glasshouse conditions, against cucumber powdery mildew, which is caused by *Podosphaera xanthii* (Choi *et al*., 2004).

It has been reported that chrysophanol exhibited inhibitory effect against *Entamoeba histolytica*, with an IC_{50} of 6.21 µg/ml (Moo *et al*., 2007).

Chrysophanol has been investigated its capability to cause chromosomal aberrations in the Chinese hamster ovary cell assay. There were no significant increases in chromosomal aberrations when chrysophanol was tested up to its limit of solubility with or without metabolic activation. The result indicates that chrysophanol had no clastogenic activity under the conditions described (Mengs *et al*., 2001).

2.8 Biosynthesis of anthraquinones

A number of natural anthraquinone derivatives are acetate-derived structures. Endocrocin (Figure 2-3) found in species of *Penicillium* and *Aspergillus* fungi are formed by folding a polyketide containing eight C_2 units to form the periphery of the carbon skeleton. Three aldol-type condensations would give a hypothetical intermediate 1, and except for crucial carbonyl oxygen in the centre ring, endocrocin results by enolization reactions, one of which involves the vinylogous enolization. The additional carbonyl oxygen must be introduced at some

stage during the biosynthesis by an oxidative process. Emodin, a metabolite of some *Penicillium* species, but also found in higher plants, e.g. *Rhamnus* and *Rumex* species, would appear to be formed from endocrocin by a simple decarboxylation reaction. This is facilitated by the adjacent phenol function. *O*-Methylation of emodin would then lead to physcion. (Dewick, 2001)

Islandicin is another anthraquinone pigment produced by *Penicillium islandicum*, and differs from emodin in two ways. One hydroxyl is missing, and a new hydroxyl has been incorporated adjacent to the methyl. Without any evidence for the sequence of such reactions, the structure of intermediate 2 shows the result of three aldol condensations and reduction of a carbonyl. A dehydration reaction, two oxidations, and a decarboxylation are necessary to attain the islandicin structure (Dewick, 2001).

In chrysophanol, aloe-emodin, and rhein, the same oxygen function is lost by reduction as in islandicin, and decarboxylation also occurs. The three compounds are interrelated by a sequential oxidation of the methyl in chrysophanol to a hydroxymethyl in aloe-emodin, and a carboxyl in rhein (Dewick, 2001).

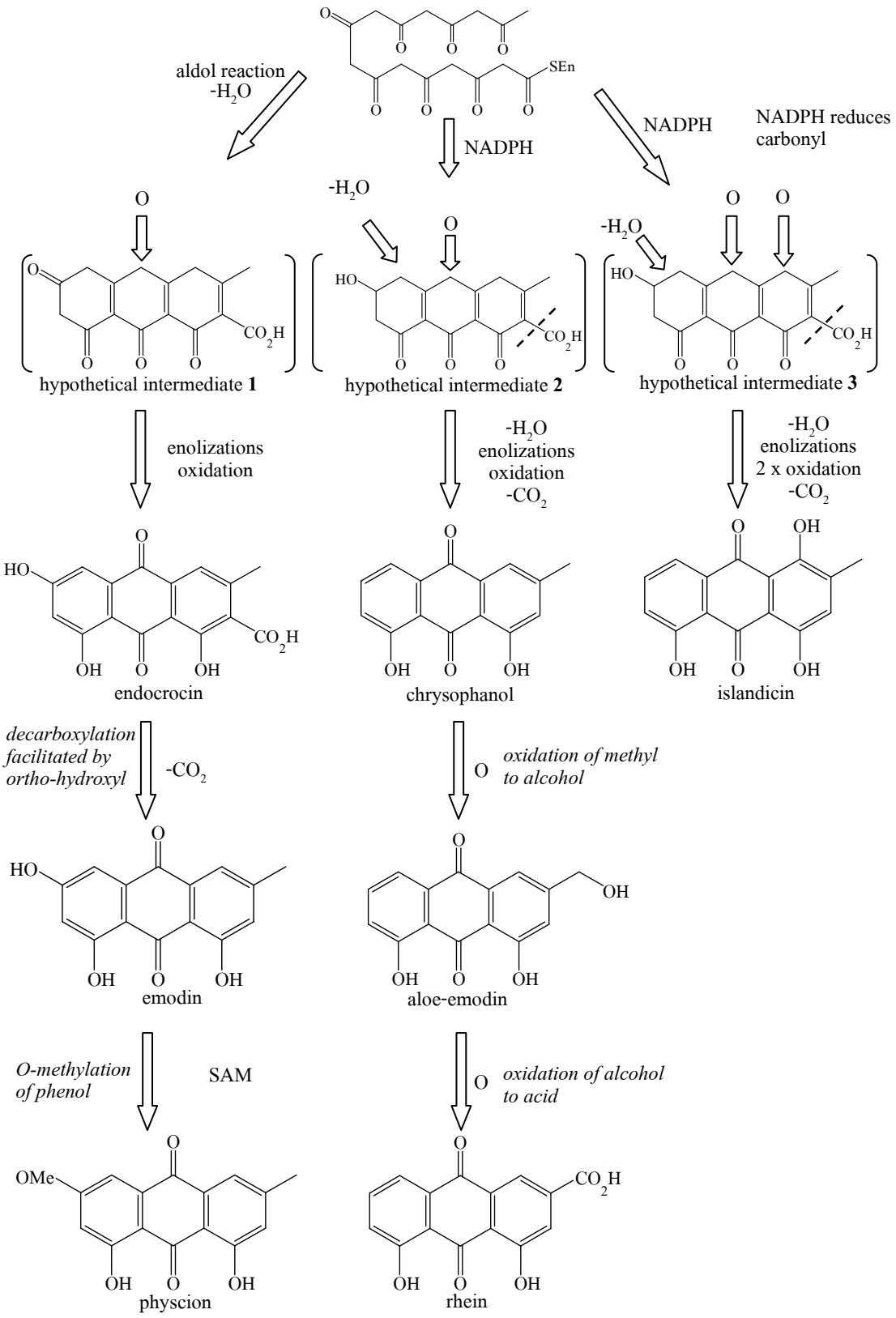


Figure 2-3 Biogenesis pathways of anthraquinones (Dewick, 2001)

2.9 Standardized of herbal extract (Tierra, 1999)

Standardized extracts arise out of the need to create a uniform product for clinical trials. Broadly speaking, there are two types. One is based on identifying and quantifying an extract to a characteristic chemical marker compound. The second identifies and concentrates one or more as active constituents, making it closer to the level of a chemical isolate. This means that other naturally occurring constituents are displaced at the expense of one or a number of compounds.

Those who support standardized extracts believe that they represent a trend towards higher technological refinement. They believe that they will provide a more consistent, stronger and more effective product backed by chemical analysis to confirm the presence and ratio quantity of one or a number of characteristic plant constituents. This will increase consumer confidence and this is ultimately good for greater acceptance of herbs by the medical establishment and the mainstream.

Certainly many of these are positive in many of these respects but there remain important critical issues to consider. Quality and effectiveness may be compromised in a number of ways:

1. For the extracts based on active constituents, the high degree of concentration causes a corresponding displacement and lack of other constituents, which in a few cases have been subsequently shown to be even more effective than the originally presumed active constituent.

2. Again for active constituent extracts, given that there may be only a partial representation of the herb's normally occurring constituents, this limits the broad range of traditionally known properties and uses of an herb in favor of a single use.

3. The use of chemical constituents as active or marker compounds creates misinformation encouraging the misuse of herbs as a substitute for drugs. This demeans in popular understanding the broader context of their use for the treatment of underlying imbalances as the cause of disease.

4. Not all herbs branded as standardized are manufactured the same. Some involve methods that are not dissimilar to a more highly refined tincture or a concentrated dried extract while others employ the use of toxic solvents that may go against the sensibilities and ethics of individuals who are attracted to the use herbal remedies as an alternative to drugs. Furthermore, different methods of standardization produce significant differences in the finished product of which the consumer is not aware.

5. The need to extract high isolates of a single biochemical constituent fosters poor harvesting and wild crafting (ecologically sound harvesting of wild herbs) where quantity is sacrificed for quality.

6. The relationship of plant to human is challenged so that people are less likely to appreciate the fact that an herb growing amongst the weeds in their garden may make as effective or an even more effective remedy than a standardized extract.

7. Since standardized extracts essentially represent a different form, it is not to be assumed that they will have the same effects as more conventional herbal products such as a non-chemically standardized tincture.

8. The promotion of standardized extracts for the treatment of a named pathology encourages marketing opportunism. This tends to distract from other herbs and products such as the use of Chinese red sage (*Salvia Milthiorrhiza*) or Tienchi ginseng (*Panax pseudoginseng*) instead of hawthorn for heart disease, chrysanthemum flowers (*Chrysanthemum morifolium*) instead of feverfew for migraine headaches and honeysuckle blossoms (*Lonicera javonica*) instead of echinacea for the common cold.

9. The technology necessary to produce truly standardized extracts as espoused by some of their leading exponents greatly changes the way herbs are handled at all stages from growth to final product. Some proposed trends such as the exclusive use of cultivated herbs over wild harvested ones, is counter to the traditional time-honored principles and practices of herbal medicine. Herbalists have always felt that herbs grown in the wild are superior to those under cultivation and by definition, wild herbs cannot be standardized. To manipulate herbs to conform to an artificial process of standardization makes them more like 'phytopharmaceutical' drugs. This in turn means that they can only be manufactured into products by well-vested pharmaceutical companies to be distributed and sold in pharmacies under prescription by medical doctors. Herbal medicine is a rigorous study and medical practice unto itself. It is presumptuous to assume that the majority of medical doctors are or ever will be qualified in their proper use. With profit as the primary motive, there is good reason to distrust pharmaceutical companies considering that it was from this sector that one may largely attribute the nearly complete suppression of herbal medicine from the mid-1920's to the late 60's. Up to recently, pharmaceutical companies were unable to cash in on the sale of herbs because they were unpatentable. With the advent of standardization, there is a pattern established where a company that is able to spend huge amounts of money on research is entitled to develop an exclusive patent for the process of extraction and standardization of an herbal product accompanied with a license to sell them on the international market.

As briefly described, by definition, a standardized herbal extract involves predetermining one or a number of biochemical constituents as either active or as marker compounds. The result involves two very distinct types of extracts.

First: a marker extract

This type establishes that a specified amount of a marker compound is present in the finished product. It must be remembered that a marker does not represent the active constituents but is selected as a biochemical constituent characteristic of the plant. In many cases,

if this process uniformly increases all plant constituents to an intended level. In general, the insoluble compounds, such as cellulose and fiber, are excluded. In some cases the concentrated extracts remain dried and powdered while in others they are mixed with a neutral material such as corn starch, and in still others, the extract is mixed with the fine granules of the whole herb. The most important distinction is that marker extracts are not based on the concentration of a proven active constituent, but are used for positive identification or to create a higher degree of uniform potency. As for the latter, it as yet remains to be proven whether this is consistent with the potency of the whole herb.

Considering the fact that all herbal preparations are to a degree an extract, a marker extract that equally regulates all constituents, retains more relationship to the traditional way herbalists use herbs. Another problem is the propensity to overly promote one use over many others that it may have. This tends to be based on a named pathology, such as feverfew for migraines, St John's Wort for depression, devil's claw for arthritis. Traditional herbal medicine uses herbs more functionally rather than for the treatment of specific pathology. Falter described the Eclectic tradition's specific indications for hypericum as including a range of conditions from spinal injuries, concussions, chronic urinary disorders, to various pains associated with bruising, injuries and puncture wounds and finally as a sedative for hysteria which may in modern usage translate to its anti-depressive properties.

There are some differences as to how each herb is treated but in general the following is a list of some of the better known marker extracts.

Examples of marker extracts are Artichoke (2-5% cynarin), Chamomile (1.2% apigenin/0.5% essential oil), Devil's claw (5% harpogosides), Echinacea (4% echinacosides), Ephedra (6-8% ephedrine/pseudoephedrine), Feverfew (2.6% parthenolides), ginseng (5-15% ginsenosides), Goldenseal (5% hydrastine), Horsechestnut (20% Aescin), Uva Ursi (20% Arbutin), Gotu Kola (10% asiaticosides), Green tea (20-50% polyphenols), Licorice (12% glycyrrhizin), St Johnswort (0.3-0.5% hypericin), Schisandra (2.6-4% schisandrins), Valerian (0.8-1% valerenic acid), and Willow (8% salicin).

Second: an active constituent extract

This regulates a specific biochemical constituent to a level that may not be naturally found in the plant. Concentrating 95% curcuminoids, for instance, in a standardized turmeric extract creates a product that while derived from the crude herb, is not expected to be naturally found concentrated at that level. This leaves only 5% of the other turmeric constituents with which the curcumin is combined.

There are at least three specific and obvious problems with pumping up one constituent of a product at the expense of its other constituents:

1. The active constituent may indeed be eventually found to be not primarily responsible for the therapeutic action of the herb, i.e. other constituents may be discovered to be more biologically active.

2. Herbs such as turmeric, milk thistle seeds and saw palmetto have many properties and uses. One of the problems with the promotion and intended use of standardized extracts in this category is that they may limit an herb's range of influence. How do we know that by excluding the complex of chemicals found in each of these herbs, they don't lose their more varied and diverse traditional functions? In fact, I would probably not use a standardized curcumin extract to promote blood circulation or warm and stimulate digestion. While Traditional Chinese Medicine (TCM) classifies it as warm (stimulating metabolism), emphasizing its anti-inflammatory curcumin content would make it more cooling (lowering metabolism).

3. There are questions about safety when concentrations of one or a number of biochemical agents are allowed to be sold as food supplements when in many cases it may really be more of a phytotherapeutic drug. One example is *Ephedra sinica* or Ma Huang (the Chinese name). As a whole plant it has been used for respiratory conditions, especially asthma. According to Dr. Andrew Weil, M.D., ephedra has been replaced in medical

practice by "pure, synthetic ephedrine," based on one of the plant's active constituents. While patients taking pure synthetic ephedrine, experience side effects of "jitteriness, insomnia and a feeling of being drained of energy all day," he points out that a tea made of the whole herb "gives similar relief from asthma, but with "none or little of ephedrine's toxicity."

4. Given the sensitivity around a small number of adverse reactions to certain herbs in the recent past, it would not take many complaints that would probably go against the herb rather than only its standardized extract. Again, not all of these issues would apply to every herb in this category.

The problem of exclusively promoting an herb as a standardized extract overlooks the range of many of its other uses. For instance, Milk thistle seed extract is specifically sold as a liver protective agent while the Eclectics according to Felter and Lloyd as quoted in Ellingwood described a much wider use. These include a dull aching pain over the spleen that passes up to the left scapula associated with pronounced debility and despondency, splenic pain with or without enlargement. Further uses indicate that it was known to improve blood circulation especially in the pelvis making it particularly useful for dysmenorrhea, amenorrhea and irregular passive uterine hemorrhages.

Examples of active constituents extracts are Gingko (24% flavoglycosides), Milk thistle (80% silymarin), Grape seed (95% polyphenols), turmeric (95% curcumin), Saw palmetto (90% free fatty acids), Green tea (60% catechins), *Cascara sagrada* (20-30% anthraquinones), Bilberry (25% anthocyanosides), Pygeum (12% phytosterols), and Kava (30-40% kavalactones).